Outcome in Cerebral Venous Sinus Thrombosis. Brain Sci. 2020;11(1):23.
doi: https://doi.org/10.3390/brainsci11010023

11. Kovács E, Bereczky Z, Kerényi A, Laczik R, Nagy V, Kovács DÁ, et al. Clinical /Investigation of Hereditary and Acquired Thrombophilic Factors in Patients with Venous and Arterial Thromboembolism. Int J Gen Med. 2023 Nov 22;16:5425-37.
doi: https://doi.org/10.2147/IJGM.S412551

12. Ponti G, Ruini C, Tomasi A. Homocysteine as a potential predictor of cardiovascular risk in patients with COVID-19. Med Hypotheses. 2020;143:109859.
doi: https://doi.org/10.1016/j.mehy.2020.109859

13. Si T, Zhang W, Fu X, Wang Y, Liu D, Wu Q. Reference intervals of homocysteine in apparently healthy Chinese Han ethnic adults. J Lab Med 2022;46(2):125-32.
doi: https://doi.org/10.1515/labmed-2021-0135

14. Keskin O, Seremet Keskin A, Nilgün Seremet. Association between low serum folic acid and vitamin B12 levels with COVID-19 prognosis. Progr Nutr [Internet]. 2022 Sep. 27 [cited 2024 Mar 4];24(3):e2022104.

15. Abildgaard A, et al. Reference intervals for plasma vitamin B12 and plasma/serum methylmalonic acid in Danish children, adults and elderly. Clinica chimica acta; international journal of clinical chemistry. 2022;525:62-8.
doi: https://doi.org/10.1016/j.cca.2021.12.015

16. Galukande M, Jombwe J, Fualal J, Baingana R, Gakwaya A. Reference values for serum levels of folic acid and vitamin B12 in a young adult Ugandan population. Afr Health Sci. 2011 Jun;11(2):240-3. PMID: 21857855; PMCID: PMC3158514.

17. Golovanova IA, Belikova IV, Lyakhova NO. [Fundamentals of medical statistics. Study guide for graduate students and clinical residents]. Poltava; 2017. 113 p. Ukrainian.

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THE EFFECT OF CELECOXIB AND PARACETAMOL ON THE FUNCTIONAL STATE OF THE CENTRAL NERVOUS SYSTEM, PAIN SENSITIVITY, AND PHYSICAL ENDURANCE IN RATS WITH ACUTE HEAT INJURY

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Ключові слова: *гостра теплова травма, інгібітори циклооксигенази, целекоксиб, парацетамол, центральна нервова система, больова чутливість, фізична витривалість*

Abstract. Effect of celecoxib and paracetamol on the functional state of the central nervous system, pain sensitivity, and physical endurance of rats with acute heat injury. Chuikova P.O., Shtrygol' S.Yu. *Acute heat injury (AHI) is a serious condition caused by an excessive increase in body temperature, usually due to prolonged exposure to high*

environmental temperatures or intense physical activity in the heat. Without timely treatment, heat stroke can lead to severe damage to the central nervous system with cerebral edema, profound disturbances in the water-salt balance and internal organs, coma and death. Since the effectiveness of drugs for the treatment of thermal injuries has not been proven, the search for new thermoprotective agents with different mechanisms of action, in particular inhibitors of the arachidonic acid cascade, is urgent. In a preliminary screening study on the AHI model in rats, it was found that among cyclooxygenase (COX) inhibitors, the highly selective COX-2 inhibitor celecoxib and the analgesic-antipyretic paracetamol are the most effective in preventing hyperthermia and improving the course of the recovery period. The purpose of this study was to determine the impact of the specified screening leaders on the functional state of the central nervous system, pain sensitivity and physical endurance in the recovery period of heat injury. The AHI model was reproduced on adult white male rats according to the previously proposed and validated method by means of a 30-minute exposure at +55°C. Animals were divided into 4 groups with 8 rats in each group: intact control, control pathology, paracetamol group and celecoxib group. Based on the results of the study, it was established that celecoxib exhibits a pronounced thermoprotective effect, probably improves the state of the central nervous system in terms of behavioral reactions and physical endurance of animals in the recovery period after acute heat injury. At the same time, paracetamol after acute heat injury does not have a distinct positive effect on the functional state of the central nervous system, moderately improves the physical endurance of rats and is inferior to celecoxib in all the studied parameters. These results open new opportunities for the development of approaches to the treatment of AHI and confirm the different effectiveness of the use of celecoxib and paracetamol in thermal injuries.

Реферат. Вплив целекоксибу та парацетамолу на функціональний стан центральної нервової системи, больову чутливість та фізичну витривалість щурів за гострої теплової травми. Чуйкова П.О., Штриголь С.Ю. *Гостра теплова травма (ГТТ) — серйозне порушення здоров'я, викликане надмірним підвищенням температури тіла, зазвичай у результаті тривалого перебування в умовах високої температури довкілля або внаслідок інтенсивної фізичної активності в спеку. Без своєчасного лікування тепловий удар може призвести до тяжких уражень центральної нервової системи (ЦНС) з набряком головного мозку, глибоких порушень з боку внутрішніх органів та водно-сольового балансу, коми та смерті. Позаяк ефективність лікарських препаратів для лікування теплових уражень не доведена, актуальним є пошук нових термопротекторних засобів з різними механізмами дії, зокрема інгібіторів каскаду арахідонової кислоти. У попередньому скринінговому дослідженні на моделі ГТТ у щурів виявлено, що з-поміж інгібіторів циклооксигенази (ЦОГ) найефективніше запобігають гіпертермії та поліпшують перебіг відновного періоду високоселективний інгібітор ЦОГ-2 целекоксиб та анальгетик-антипіретик парацетамол. Метою цього дослідження стало визначення впливу зазначених лідерів скринінгу на функціональний стан ЦНС, больову чутливість та фізичну витривалість у відновному періоді теплової травми. Модель ГТТ відтворювали на дорослих білих щурах-самцях за запропонованою раніше валідованою методикою шляхом 30-хвилинної експозиції за +55°C. Тварини були розподілені на 4 групи по 8 щурів у кожній групі: інтактний контроль, контрольна патологія, група парацетамолу та група целекоксибу. За результатами дослідження встановлено, що целекоксиб виявляє виражену термопротекторну дію, вірогідно поліпшує стан ЦНС за поведінковими реакціями та фізичну витривалість тварин у відновному періоді після ГТТ. Водночас парацетамол після ГТТ не чинить вираженого позитивного впливу на функціональний стан ЦНС, помірно поліпшує фізичну витривалість щурів та поступається за всіма дослідженими показниками целекоксибу. Ці результати відкривають нові можливості для розробки підходів до лікування ГТТ та демонструють неоднакову ефективність використання целекоксибу та парацетамолу за теплових уражень.*

Acute heat injury (AHI) from exposure to high-temperature environments is accompanied by an excessive increase in body temperature, surpassing the body's ability to dissipate heat. This condition is life-threatening and requires immediate medical attention [1]. The issue of AHI is becoming increasingly relevant for several reasons. Global warming raises the average temperatures on the planet, increasing the frequency and intensity of heat waves and, consequently, the risk of AHI [2]. Lifestyle changes and increased physical activity among the population also contribute to the rising frequency of AHI. The growing popularity of outdoor recreation and sports is not always accompanied by adherence to safety measures during training in hot weather, leading to more frequent heat strokes among athletes and outdoor enthusiasts [3]. Socio-economic conditions also play a significant role. People who work outdoors or in non-air-conditioned environments, especially in low- and middle-income countries, are at higher risk. This category includes agricultural workers, construction workers, transport workers, and others [4, 5]. Finally, demographic changes, particularly the aging population, also contribute to the increase in AHI cases, as older individuals are more vulnerable to high temperatures due to physiological changes and the presence of chronic diseases [6].

Excessively high external temperatures in humans can lead to an increase in body temperature above 40°C, changes in mental state (confusion, disorientation, or unconsciousness), lack of sweating, redness and dryness of the skin, rapid heart rate, and

breathing. Without proper and timely treatment, heat stroke can cause severe damage of central nervous system (CNS) with brain swelling, profound disruptions in water-salt balance, damage to internal organs, coma, and death [7].

Thermoprotective agents are needed for the treatment of AHI. The effectiveness of pharmaceutical treatments for heat injuries has not been proven (except for medications for treating seizures and edema, correcting water-salt balance) [8]. This justifies the relevance of searching for thermoprotectors with different mechanisms of action [7, 8, 9]. The inflammatory cascade of arachidonic acid plays an important role in the pathogenesis of AHI, attracting attention to cyclooxygenase (COX) inhibitors with different selectivity of action as potential thermoprotectors.

In previous screening studies on a rat model of AHI, we found a pronounced thermoprotective effect of the nonsteroidal anti-inflammatory drug (NSAID) celecoxib – a highly selective COX-2 inhibitor, and the analgesic-antipyretic paracetamol – a non-selective COX inhibitor with predominantly central action. Their effect was manifested by a lesser increase in body temperature of the animals compared to several other NSAIDs – acetylsalicylic acid, diclofenac sodium, nimesulide, etoricoxib [10]. It is important to thoroughly characterize the quality of the thermoprotective effect from the perspective of the state of individual organs and systems affected by high environmental temperatures. The aim of this study was to determine the impact of the mentioned screening leaders on the functional state of the CNS, pain sensitivity, and physical endurance during the recovery period after heat injury.

MATERIALS AND METHODS OF RESEARCH

The animals were kept on a standard vivarium diet with free access to water, constant humidity, and a temperature regime of +22-23°C. All experiments were approved by the Bioethics Committee of the National University of Pharmacy (excerpt from the meeting protocol No. 12 dated January 10, 2024) and conducted in accordance with the requirements of the European Convention "For the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986, as amended in 1998), in compliance with the Law of Ukraine No. 3446–IV dated February 21, 2006, as amended "On the Protection of Animals from Cruelty" and the European Union Directive 2010/63 EU "On the Protection of Animals Used for Scientific Purposes."

The model of AHI was reproduced in male white rats weighing 250-300 g according to a previously validated methodology, which established the duration and temperature of heat exposure that does not cause animal death and meets current bioethical standards but allows for the creation of pronounced hyperthermia [10]. For this purpose, the animals were placed in a thermostat at a temperature of +55°C for 30 minutes. Rectal temperature was monitored before and at the end of the heat exposure. An electronic thermometer Gamma Thermo Base was used for precise temperature measurement.

In the acute period of AHI, 10-15 minutes after heat exposure, sequential testing was started. Initially, in the open field test, motor activity (number of squares crossed) and exploratory activity (number of vertical rears and exploratory nose-pokes, their sum), emotional responses (number of grooming acts), and their autonomic accompaniment (number of fecal boli, urinations) were assessed separately and in total, calculating the sum of all activities [11, 12]. Muscle tone and coordination of animal movements were evaluated using the rotating rod test (speed 10 revolutions per minute) [13]. Following this, pain sensitivity and the effect of the tested substances were assessed using the "Hot Plate" test (surface temperature +54°C), taking into account the latency time of the typical response – licking the hind paw [14]. To avoid burns, the duration of animal exposure to the hot plate did not exceed 60 seconds.

The final stage (30 minutes after heat exposure) was the evaluation of physical endurance using the forced swimming test with a load (10% of body weight at the root of the tail). The animals were placed in a pool with a diameter of 50 cm, a depth of 60 cm, and side walls 10 cm high, which prevents escape, with room temperature water (21-22°C). The swimming time until exhaustion, when the rats submerged underwater and could not surface for 10 seconds, was measured [15].

The rats were randomly divided into 4 groups, which were homogeneous in mass and body temperature at the beginning of the study. Group 1 – intact control, n=8; group 2 – control pathology (AHI), n=8; group 3 – paracetamol at a dose of 125 mg/kg + AHI, n=8; group 4 – celecoxib at a dose of 8.4 mg/kg + AHI, n=8. Celecoxib ("Celebrex", capsules 200 mg, "Pfizer", USA) and paracetamol (Paracetamol, capsules 500 mg, "Zdorovye", Ukraine) were used. The drugs, in the form of a suspension with Tween-80, were administered orally using a probe in a volume of 2 ml/kg 60 minutes before the start of heat exposure. Animals in the intact control and control pathology groups received water in a similar volume.

Statistical processing of the results was performed using the licensed software Statistica 10.0 (StatSoft Inc., serial number STA999K347156-W). The distribution of the sample data was assessed using the Shapiro-Wilk test. As the distribution was not normal,

the Kruskal-Wallis test was used to evaluate the statistical significance of differences between several independent groups. To determine which specific groups had significant differences, post-hoc comparisons were conducted using the Mann-Whitney test. Results were presented as medians, 25% and 75% percentiles (Me[Q25;Q75]), arithmetic means with standard errors (M±m), and percentages. For accounting results in an alternative form (presence/absence of a feature), Fisher's angular transformation was used. Differences were considered statistically significant at $p<0.017$. The patterns of relationships between individual indicators were analyzed using Spearman's correlation coefficient [16].

RESULTS AND DISCUSSION

The baseline body temperature in all groups was nearly identical and within the physiological norm (36.1-38.1°C). After heat exposure, all animals exhibited an increase in temperature compared to the initial state. Hyperthermia was particularly pronounced in the control pathology group, with an average increase reaching 5.11°C (up to 42.41°C), indicative of severe heat stroke (HS). Celecoxib significantly reduced the severity of hyperthermia (average temperature increase +2.48°C, $p<0.01$ compared to the control pathology). Paracetamol tended to prevent overheating (average body temperature increase was 4.35°C) without statistically significant differences compared to the control pathology group (Table 1).

In the open field test during the early recovery period after AHI, rats in the control pathology group exhibited a significant decrease in exploratory activity and autonomic support of emotional reactions compared to the intact control group (Table 2). In the remaining subtests, as well as in the sum of all activities, there was a trend towards decreased values for all measures. These results indicate CNS suppression due to heat stroke.

Table 1

Changes in body temperature of rats during acute heat stroke, M±m, Me[Q25;Q75]

Group, number of animals	Body temperature, °C		Overall increase, °C
	initial	at 30 min of heat exposure	
Intact control (n=8)	**37.10±0.39** **37.1[36.2; 37.8]**	—	—
Control pathology (n=8)	**37.30±0.26** **37.2[37.0; 37.6]**	**42.41±0.14**** **42.6[42.2; 42.7]**	**5.11±0.16** **5.2 [5.0; 5.4]**
Paracetamol (n=8)	**37.38±0.14** **37.3[37.3; 37.6]**	**41.73±0.43**** **41.9[40.9; 42.7]**	**4.35±0.38** **4.5 [4.0; 4.9]**
Celecoxib (n=8)	**37.05±0.19** **37.2[36.7; 37.3]**	**39.53±0.24**** **39.6[39.2; 39.9]**	**2.48±0.26$^{\#\#}$** **2.7 [2.2; 3.0]**

Notes: n – number of animals in the group; ** – significant differences from the baseline, $p<0.01$; ##– significant differences from the control pathology group, $p<0.01$.

In the paracetamol group, behavioral reactions also worsened. Exploratory activity significantly decreased in terms of the total sum of indicators and the sum of emotional reaction indicators. These rats were twice as impaired in overall activities compared to intact animals (Table 2).

Celecoxib demonstrated a significant positive impact on behavioral reactions in the open field test following acute heat trauma. The celecoxib group did not lag behind the intact control animals in any indicator and significantly outperformed the control pathology group in terms of exploratory activity ($p<0.05$). Furthermore, celecoxib tended to increase the motor activity of the animals, particularly in comparison with the control pathology group.

Based on the results of the correlation analysis, the relationship between body temperature and the sum of all activities in the open field test differs significantly. In intact animals, this relationship is a strong negative correlation, meaning that higher body temperature is associated with lower activity ($r= -0.814$, $p=0.018$). In the Heat Tolerance Test (HTT), this relationship weakens in the control pathology group and in the presence of paracetamol ($r= -0.357$ and $r= -0.336$, respectively) and does not reach significant levels. However, under the influence of celecoxib, the correlation becomes a positive moderate one ($r=0.402$) and is also not significant.

Table 2

Effect of paracetamol and celecoxib on rat behavior in the open field test during early recovery after acute heat injury, M±m, Me[Q25;Q75]

Group, number of animals		Intact control (n=8)	Acute heat injury		
			control pathology (n=8)	paracetamol (n=8)	celecoxib (n=8)
Locomotor activity (squares crossed)		29.25±7.60 23.5 [15.3;38.3]	22.0±8.11 9.0 [7.3;39.5]	18.63±8.29 12.5 [2.5;25.3]	33.13±5.09 38.0 [33.8;39.0]
Motor and exploratory activity	Rearings	9.0±1.40 9.0 [5.8;10.8]	3.0±1.29 2.0 [0.8;3.8]**	4.25±1.75 4.0 [0; 6.3]	5.88±1.13 6.5 [4.8;7.3]
	Exploratory nose-pokes	1.38±0.64 1.0 [0;2.0]	0.38±0.28 0 [0; 0.3]	0.88±0.32 1.0 [0; 1.3]	2.75±0.75 3.0 [1.0; 4.0]##
	The sum	10.38±1.60 10.5 [8.0;12.0]	3.38±1.19 2.0 [2.0;3.8]**	5.13±1.96 4.5 [0.8;8.0]*	8.63±1.65 10.0 [7.3;11.0]#
Emotional responses and their autonomic accompaniment	Groomings	1.0±0.20 1.0 [1.0;1.0]	0.88±0.37 0.5 [0; 2.0]	0.63±0.35 0 [0;1.3]	0.75±0.27 1.0 [0; 1.0]
	Fecal boli	0.88±0.43 0.5 [0;1.3]	0±0 0 [0; 0]	0±0 0 [0; 0]	1.13±0.55 0.5 [0;2.0]
	Urinations	0.38±0.20 0 [0;1.0]	0±0 0 [0; 0]	0±0 0 [0; 0]	0±0 0 [0; 0]
	The sum	2.25±0.44 2.0 [2.0;2.0]	0.88±0.37 0.5 [0; 2.0]*	0.63±0.35 0 [0;1.3]**	1.88±0.62 1.5 [0.8;3.3]
The sum of all activities		41.88±8.83 34.5 [23.8; 51.5]	26.25±9.16 13.0 [10.0; 44.0]	24.38±9.78 18.0 [2.8; 39.0]	43.63±6.93 50.5 [41.8;54.3]

Notes: n – number of animals in the group; * – statistically significant differences from intact control, p<0.05; ** – p<0.01; # – statistically significant differences from control pathology, p<0.05; ## – p<0.01.

According to the rotarod test results, acute heat trauma did not adversely affect the muscle tone or coordination of the animals' movements: there were no statistically significant differences between groups in the number of animals falling off the rod at different time intervals (Table 3).

Table 3

Effect of paracetamol and celecoxib on muscle tone and motor coordination in rats using the rotarod test during the early recovery period after acute heat trauma, M±m, Me[Q25;Q75]

Group, number of animals	The number of rats that fell from the rod, abs. / %		
	up to 30 s	up to 1 min	up to 5 min
Intact control (n=8)	3/37.5	2/25	3/37.5
Control pathology (n=8)	3/37.5	3/37.5	2/25
Paracetamol (n=8)	4/50	2/25	2/25
Celecoxib (n=8)	3/37.5	2/25	3/37.5

Notes: n – number of animals in the group; In the numerator, absolute number of animals; in the denominator, percentage (%).

Correlation analysis revealed that body temperature does not play a significant role in motor coordination, neither in intact rats nor in the AHI. The relationship between rectal temperature and the time to fall from

the rotating rod is non-significant and generally weak: in the intact control group, r= -0.201; in the control pathology group, r=0.080; in the paracetamol group, r= -0.366; and under celecoxib, r= -0.163.

Pain sensitivity was assessed using the acute thermal stimulation of the paw model in the "hot plate" test (Table 4). In the control pathology group, the reaction time to pain increased slightly (at a trend level). Both celecoxib and, particularly, paracetamol exhibited a statistically significant analgesic effect.

Differences in the correlation between pain sensitivity and body temperature were observed. In intact animals, the relationship between rectal temperature and the reaction time to thermal stimulation of the paw is weakly positive and non-significant (r=0.335). After the Heat Tolerance Test (HTT) in the control pathology group, this relationship disappears completely (r=0.056). However, under the influence of both COX inhibitors, it becomes strong: with paracetamol, r=0.794 (p=0.023), and with celecoxib, r=0.887 (p=0.0005). This indicates that with the use of both thermal protectors, especially celecoxib, but not in the absence of pharmacological correction, pain sensitivity to thermal stress decreases directly proportional to body temperature.

Table 4

Effect of paracetamol and celecoxib on pain sensitivity in rats using the hot plate test during the early recovery period after acute heat trauma, M±m, Me[Q25;Q75]

Group, number of animals	Back paw licking time, s
Intact control (n=8)	**12.89±0.77** **12.1 [11.2; 15.2]**
Control pathology (n=8)	**16.83±2.59** **13.8 [11.9; 21.2]**
Paracetamol (n=8)	**30.23±7.42*** **22.3 [14.6; 45.4]**
Celecoxib (n=8)	**23.34±3.32**** **20.1 [15.2; 31.0]**

Notes: n – number of animals in the group; Statistically significant differences from intact control: * – p<0.05, ** – p<0.01.

The results of the study on physical endurance in rats using the forced swimming test with a load after acute thermal trauma (Table 5) showed that the swimming duration in the control pathology group decreased by 33% compared to the intact control (p<0.01).

Table 5

Effect of paracetamol and celecoxib on physical endurance in rats using the forced swimming test during the early recovery period after acute heat trauma, M±m, Me[Q25;Q75]

Group, number of animals	Number of rats remaining on the water surface				Duration of swimming, s
	up to 30 s	0.5-1 min	1-2 min	over 2 min	
Intact control (n=8)	**0** **(0%)**	**0** **(0%)**	**6** **(75%)**	**2** **(25%)**	**103.38±8.42** **96.0 [89.5; 114.3]**
Control pathology (n=8)	**0** **(0%)**	**3**** **(37.5%)**	**4** **(50%)**	**1** **(12.5%)**	**69.38±8.58**** **62.5 [57.3; 73.0]**
Paracetamol (n=8)	**1** **(12.5%)**	**1** **(12.5%)**	**5** **(62.5%)**	**1** **(12.5%)**	**90.63±15.47** **97.5 [65.0; 108.3]**
Celecoxib (n=8)	**0** **(0%)**	**0**$^{\#\#}$ **(0%)**	**5** **(62.5%)**	**3** **(37.5%)**	**113.50±7.72**$^{\#\#}$ **106.5 [101.0; 125.8]**

Notes: n – number of animals in the group; ** – statistically significant differences from intact control, p<0.01; $^{\#\#}$ – statistically significant differences from control pathology, p<0.01.

The analysis of the dynamics of the test shows that in the control pathology group, over a third of the rats remained on the water surface for only 0.5-1 minute. This significantly ($p<0.01$) falls short compared to the intact control group, where all animals stayed on the water surface for at least 1 minute, and a quarter of the rats remained for over 2 minutes.

In the paracetamol group, physical endurance after acute heat trauma worsened, with swimming time tending to be 12% shorter than in the intact control group, and complete exhaustion occurred earlier (within 1 minute) in a quarter of the cases.

However, under the influence of celecoxib, the time to exhaustion not only did not decrease but tended to increase by 9% compared to the intact control group. Furthermore, celecoxib significantly ($p<0.01$) increased the swimming duration by 63% compared to the control pathology group. This indicates a positive effect of both thermoprotective agents on preserving physical endurance.

The analysis of the relationship between rectal temperature and physical endurance revealed a strong positive correlation in intact rats ($r=0.837$, $p=0.013$). It is possible that a healthy animal in the swim test with a load can utilize body thermal energy for physical work, so as the baseline body temperature increases, the duration of swimming to exhaustion also increases. However, despite the elevated body temperature after the AHI without the use of thermal protectors, this relationship disappears completely (in the control pathology group, $r=0.03$). Under the influence of COX inhibitors, this correlation turns into a moderate negative one ($r= -0.535$ with paracetamol) or a weak negative one ($r= -0.182$ in the celecoxib group), although the marker of physical endurance-time to exhaustion in the swim test-shows no significant differences from that of intact rats in both groups. This phenomenon cannot be explained by impaired motor coordination, as the results of the rotarod test show no changes in this respect. It is likely that the opposite direction of the studied relationship reflects characteristics of energetic metabolism, which requires further specialized investigation.

AHI is a dangerous condition leading to significant alterations in CNS function. Among the primary symptoms of CNS damage are headaches, dizziness, delirium, ataxia, seizures, and coma, which may accompany encephalopathy, brain edema, and other neurological disorders [17]. The mechanisms of CNS damage due to heat injury may be associated with increased activity of COX and LOX enzymes, as they play a critical role in inflammation and oxidative stress linked to neurodegenerative processes [18].

According to limited literature data, there are at least two mechanisms by which COX activity is increased during the AHI. The first mechanism [19], described as early as 1989, involves a response to elevated temperatures where cells alter their lipid composition and membrane fluidity. This process results in the release of arachidonic acid from phospholipids through the action of phospholipases.This, in turn, induces the formation of eicosanoids – prostaglandins, thromboxane, prostacyclin, and leukotrienes, that are involved in the inflammatory response and disruption of circulation. The second mechanism is related to the activation of HSF1 (Heat Shock Factor 1), a transcription factor that plays a key role in the cellular response to heat stress. HSF1 not only induces heat shock proteins but also activates COX-2 release in cells [20].

These observations suggest that heat stress can coordinate the entire eicosanoid synthesis cascade responsible for the symptoms of CNS damage in HS. However, pharmacological aspects of HS correction and effective target interventions remain insufficiently explored, and treatment remains largely symptomatic.

Celecoxib, a highly selective COX-2 inhibitor [21], reduces inflammation by decreasing the release of eicosanoids, particularly pro-inflammatory prostaglandins, and has demonstrated a pronounced positive impact on CNS function in HS in our study.

In contrast, paracetamol, an analgesic-antipyretic, does not have as strong an effect on COX-2 inhibition mechanisms. However, it exhibits central analgesic action, which may be explained by the inhibition of COX-3, responsible for prostaglandin synthesis in the CNS rather than the periphery [22].

Celecoxib and paracetamol have different effects on COX activity in the CNS, which may account for their distinct effects in HS. As indicated by our study results, celecoxib more effectively improves CNS function (preventing suppression of exploratory reactions and normalizing autonomic responses to emotional reactions) and physical endurance, whereas paracetamol is somewhat more effective in reducing pain sensitivity. This makes both drugs promising for the comprehensive treatment of HS, considering the specific clinical needs of each patient. Differences in the correlation between body temperature and behavioral responses, pain sensitivity, and physical endurance in the presence of the investigated drugs may reflect the specific mechanisms of action and the nature of the effects of each drug on the course of the AHI.

Thus, our results open new opportunities for developing therapeutic approaches to the treatment of AHI conditions and highlight the differing effectiveness of celecoxib and paracetamol in thermal injuries. Future research should focus on elucidating the neurochemical mechanisms through which COX inhibitors of varying selectivity affect the functional state of the central nervous system under AHI conditions.

CONCLUSIONS

1. On the acute heat injury model, the selective COX-2 inhibitor celecoxib (8.4 mg/kg) effectively prevents hyperthermia in rats when used prophylactically, surpassing the analgesic-antipyretic paracetamol (125 mg/kg) as a thermoprotector. This difference in efficacy may reflect the distinct mechanisms of action of each drug.
2. The functional state of the CNS in animals with acute heat injury deteriorates in the open field test, characterized by suppression of exploratory behavior, emotional reactions, and their autonomic responses, with a significant reduction in physical endurance in the forced swimming test. Celecoxib improves the CNS state (preventing the suppression of exploratory reactions and autonomic responses in the open field test) and restores physical endurance to the level of intact animals. Paracetamol does not improve behavioral reactions in the open field but prevents a decline in physical endurance.
3. Muscle tone and movement coordination in animals after acute heat injury in the rotarod test remain virtually unchanged, with neither celecoxib nor paracetamol affecting these parameters.
4. Pain sensitivity in rats with acute heat injury in the hot plate test is tendentially reduced. Celecoxib and especially paracetamol maintain their inherent analgesic effects under these conditions.
5. In terms of positive impact on the functional state of the CNS and physical endurance during acute heat injury, paracetamol is less effective compared to celecoxib.

Contributions:

Chuykova P.O. – investigation, writing – original draft

Shtrygol' S.Yu. – conceptualization, methodology, investigation, writing – review and editing

Recommendations. Our study lays the foundation for further research and development in the treatment of acute heat injury. Specifically, it is advisable to investigate the effects of the leading thermoprotective agents, celecoxib and paracetamol, on visceral systems, hemostasis, and energy and fluid-electrolyte balance, among other aspects.

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Conflict of interest. The authors declare that they have no conflict of interest.

REFERENCES

1. Garcia CK, Renteria LI, Leite-Santos G, Leon LR, Laitano O. Exertional heat stroke: pathophysiology and risk factors. BMJ Med. 2022 Oct 11;1(1):e000239. doi: https://doi.org/10.1136/bmjmed-2022-000239
2. Stillman JH. Heat Waves, the New Normal: Summertime Temperature Extremes Will Impact Animals, Ecosystems, and Human Communities. Physiology (Bethesda). 2019 Mar 1;34(2):86-100. doi: https://doi.org/10.1152/physiol.00040.2018
3. Périard JD, DeGroot D, Jay O. Exertional heat stroke in sport and the military: epidemiology and mitigation. Exp Physiol. 2022 Oct;107(10):1111-21. doi: https://doi.org/10.1113/EP090686
4. Kiatkitroj K, Arphorn S, Tangtong C, Maruo SJ, Ishimaru T. Risk factors associated with heat-related illness among sugarcane farmers in Thailand. Ind Health. 2022 Oct 1;60(5):447-58. doi: https://doi.org/10.2486/indhealth.2021-0161
5. How V, Singh S, Dang T, Fang Lee L, Guo HR. The effects of heat exposure on tropical farm workers in Malaysia: six-month physiological health monitoring. Int J Environ Health Res. 2023 Apr;33(4):413-29. doi: https://doi.org/10.1080/09603123.2022.2033706
6. Alari A, Letellier N, Benmarhnia T. Effect of different heat wave timing on cardiovascular and respiratory mortality in France. Sci Total Environ. 2023 Sep 20;892:164543. doi: https://doi.org/10.1016/j.scitotenv.2023.164543
7. Bauman J, Spano S, Storkan M. Heat-Related Illnesses. Emerg Med Clin North Am. 2024 Aug;42(3):485-92. doi: https://doi.org/10.1016/j.emc.2024.02.010
8. Knapik JJ, Epstein Y. Exertional Heat Stroke: Pathophysiology, Epidemiology, Diagnosis, Treatment, and Prevention. J Spec Oper Med. 2019 Summer;19(2):108-16. doi: https://doi.org/10.55460/5P2Q-1MBQ
9. Sorensen C, Hess J. Treatment and Prevention of Heat-Related Illness. N Engl J Med. 2022;387(15):1404-13. doi: https://doi.org/10.1056/NEJMcp2210623
10. Chuykova P, Shtrygol' S, Taran A, Yudkevych T, Lebedinets I, Oklei D. Acute heat trauma model in rats, gender-dependent thermoresistance, and screening of potential thermoprotectors. ScienceRise: Pharmaceutical Science. 2024 Apr;2(48):4-11. doi: https://doi.org/10.15587/2519-4852.2024.301620
11. Hock FJ, editor. Drug Discovery and Evaluation: Pharmacological Assays. B.: Springer Berlin Heidelberg; 2014. 2071 p.
12. Koval A, Shtrygol' S. 1-(5-Aetyl-3-methyl-6-phenyl-5h-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazin-7-yl)-ethanone: dose-dependence of analgesic effect, lack of

opioidergic mechanism of action, effect on behavioral reactionsand acute toxicity. Acta Medica Leopoliensia. 2023;29(3-4):192-203.
doi: https://doi.org/10.25040/aml2023.3-4.192

13. Bonm AV, Elezgarai I, Gremel CM, Viray K, Bamford NS, Palmiter RD, et al. Control of exploration, motor coordination and amphetamine sensitization by cannabinoid CB1 receptors expressed in medium spiny neurons. Eur J Neurosci. 2021 Aug;54(3):4934-52. doi: https://doi.org/10.1111/ejn.15381

14. Karna SR, Kongara K, Singh PM, Chambers P, Lopez-Villalobos N. Evaluation of analgesic interaction between morphine, dexmedetomidine and maropitant using hot-plate and tail-flick tests in rats. Vet Anaesth Analg. 2019 Jul;46(4):476-82.
doi: https://doi.org/10.1016/j.vaa.2018.12.009

15. Armario A. The forced swim test: Historical, conceptual and methodological considerations and its relationship with individual behavioral traits. Neurosci Biobehav Rev. 2021 Sep;128:74-86.
doi: https://doi.org/10.1016/j.neubiorev.2021.06.014

16. Habibzadeh F. Data Distribution: Normal or Abnormal? Journal of Korean medical science. 2024 Jan 22;39(3):e35.
doi: https://doi.org/10.3346/jkms.2024.39.e35

17. Zhang Z, Wu X, Zou Z, Shen M, Liu Q, Zhangsun Z, et al. Heat stroke: Pathogenesis, diagnosis, and current treatment. Ageing Res Rev. 2024 Jul 8;100:102409. doi: https://doi.org/10.1016/j.arr.2024.102409

18. Phillis JW, Horrocks LA, Farooqui AA. Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders. Brain Res Rev. 2006 Sep;52(2):201-43.
doi: https://doi.org/10.1016/j.brainresrev.2006.02.002

19. Calderwood SK, Bornstein B, Farnum EK, Stevenson MA. Heat shock stimulates the release of arachidonic acid and the synthesis of prostaglandins and leukotriene B4 in mammalian cells. J Cell Physiol. 1989 Nov;141(2):325-33.
doi: https://doi.org/10.1002/jcp.1041410214

20. Rossi A, Coccia M, Trotta E, Angelini M, Santoro MG. Regulation of cyclooxygenase-2 expression by heat: a novel aspect of heat shock factor 1 function in human cells. PloS One. 2012;7(2):e31304.
doi: https://doi.org/10.1371/journal.pone.0031304

21. Cruz JV, Rosa JMC, Kimani NM, Giuliatti S, Dos Santos CBR. The Role of Celecoxib as a Potential Inhibitor in the Treatment of Inflammatory Diseases – A Review. Curr Med Chem. 2022;29(17):3028-49.
doi: https://doi.org/10.2174/0929867328666210910125229

22. Przybyła GW, Szychowski KA, Gmiński J. Paracetamol – An old drug with new mechanisms of action. Clin Exp Pharmacol Physiol. 2021 Jan;48(1):3-19. doi: https://doi.org/10.1111/1440-1681.13392

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